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OBSERVATIONS ON THE SYMBIOSIS WITH ZOOXANTHELLAE AMONG THE TRIDACNIDAE (MOLLUSCA, BIVALVIA).

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Abstract

The symbiosis with zooxanthellae among tridacnid bivalve molluscs has been examined in order to assess the morphological relation of the algae to the hosts' tissues and the dynamics of the interactions between the algae and the clams.

Light and electron microscopic evidence indicates that the zooxanthellae located in the haemal sinuses of the hypertrophied siphon are intercellular in all six tridacnid species studied. The algae pass from the siphonal tissues to the alimentary tract from which they are voided as feces. Many of the defecated symbionts are morphologically intact and photosynthetically functional. The concretions in the kidneys of tridacnids, previously thought to be the indigestible remains of zooxanthellae, are identified as phosphorite deposits, which are not unique to tridacnids.

Analyses of photosynthesis-radiant flux relations in *Tridacna maxima* indicate that over 24 h the algae produce more oxygen than is consumed by the association, and estimates suggest that the algae may contribute more than 50% of the animals' metabolic carbon requirements.

Introduction

Tridacnid bivalves harbor endosymbiotic dinoflagellates, commonly referred to as zooxanthellae, within the haemal sinuses of the hypertrophied siphon (Brock, 1888; Boschma, 1924; Yonge, 1936, 1953; Mansour, 1946a, b, c; Kawaguti, 1966; Fankboner, 1971; Goreau *et al.*, 1973). The only other recorded association of bivalves with dinoflagellates is the heart shell *Corculum cardissa*, which like the tridacnids is a member of the superfamily Cardiacea (Kawaguti, 1950, 1968).

Strong experimental evidence indicates that photosynthetic products of the algae are translocated to and used by the clams in a variety of anabolic and catabolic processes (Muscatine, 1967; Goreau *et al.*, 1973; Trench, 1979).

Morphologic and metabolic relations between the animals and the algae they harbor are unclear. According to Yonge (1936, 1953), the algae in the siphonal tissues, "invariably contained in blood amoebocytes," are periodically digested by the amoebocytes after transport to the viscera. The indigestible remains of the algae were hypothesized to accumulate in the enlarged kidneys (see Goreau *et al.*, 1973; Morton, 1978). However, other investigators have reported that the algae in the siphonal tissues are intercellular (Brock, 1888; Boschma, 1924; Mansour,

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Abbreviations: DCMU = (3[3,4 dichlorophenyl]-1,1 dimethyl urea, P_{max} = maximum net photosynthetic rate, K_m = radiant flux intensity at half-maximal photosynthetic rate, P_{gross} = gross photosynthesis (P_{net} + R), R = respiration, $P_{gross}/R = (P_{net} + R)/R$, PQ = photosynthetic quotient, RQ = respiratory quotient.

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1946a, b, c; Kawaguti, 1966; Bishop *et al.*, 1976). While Kawaguti (1966) and Fankboner (1971) agree that in the siphonal tissues the algae are intercellular, they suggest that in the viscera they are contained in and digested by amoebocytes (see also Morton, 1978). Fankboner (1971) presented electron microscopic histochemical evidence which he interpreted as demonstrating lysosomal digestion of the algae in the amoebocytes in the viscera. Yet Ricard and Salvat (1977) found live zooxanthellae in the feces of *T. maxima.*

On the question of the photosynthetic capabilities of the intact association, Yonge (1936) concluded that less oxygen was produced in the light by photosynthesis than was consumed in darkness, implying that the photosynthetic contribution of the algae was insufficient to offset the respiratory requirements of the consortium. In contrast, Wells *et al.* (1973) and Jaubert (1977) found net oxygen production by *Tridacna.*

Our studies resolve the conflicting information cited above, and indicate that:

(1) the algal symbionts in the siphonal tissues of *Tridacna maxima, T. crocea, T. squamosa, T. derasa, T. gigas,* and *Hippopus hippopus* are intercellular, and lie free in the haemal sinuses;

(2) the algae pass from the siphonal tissues to the gut, where they are voided in the feces;

(3) many such defecated symbionts are morphologically intact, photosynthetically active, and can be cultured;

(4) indigestible remains of symbiotic algae do not accumulate in the kidneys; indeed, the concretions found there are identified as calcium phosphate;

(5) the oxygen produced in daylight by *T. maxima*, even on cloudy days, exceeds that which is consumed at night, indicating net production by the association.

Materials and Methods

Collection of specimens

Tridacnid clams were collected by diving off the reefs of the Republic of the Philippines; Enewetak Atoll, Marshall Islands; and Belau, Western Caroline Islands. Great care was taken when collecting to minimize damage and trauma, particularly with respect to the burrowing species. In the Philippines, animals were maintained shipboard on the *R. V. Alpha Helix*. Facilities at Enewetak were provided by the Mid Pacific Marine Laboratory, and in Belau by the Micronesia Mariculture Demonstration Center on Malakal. All animals were maintained in running sea water at ambient temperature, insolation, and salinity.

Histology

Whole small specimens were fixed intact in Bouin's or Carnoy's fixative for 24–48 h. The calcium carbonate remaining in the shell was removed by decalcification in repeated changes of 50% acetic acid. Specimens were dehydrated in methyl cellusolve (ethylene glycol monomethyl ether), cleared in xylene and dioxane, and embedded in Paraplast (Trench, 1974). Transverse serial sections 5–7 μm thick were mounted on glass slides and stained using Mallory tripple stain, haematoxylin and eosin, toluidine blue, haematoxylin and fast green FCF, Mallory phosphotungstic acid-haematoxylin, or the periodic acid-Schiff stain. The stained sections were examined and photographed with an Olympus Vanox microscope.

For ultrastructural studies, small pieces of tissues and fecal pellets were fixed

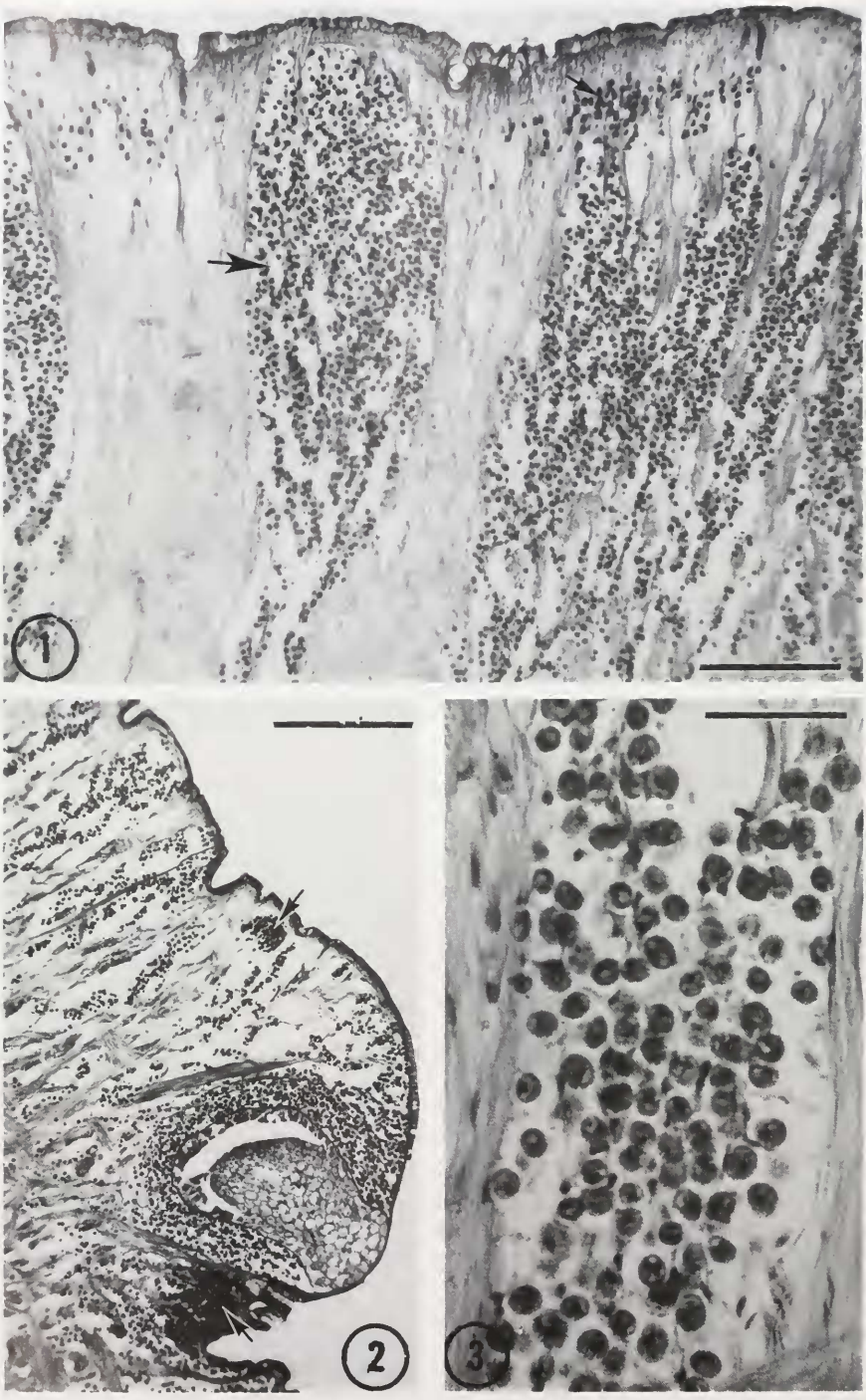

FIGURE 1. Light micrograph of a Paraplast-embedded section through the hypertrophied siphon of *T. gigas*. Heavy arrow indicates the haemal sinus containing the algae. Small arrow indicates the clusters of iridocytes. Scale bar = 200 μm.

in 6% cacodylate-buffered (pH 7.4) glutaraldehyde or in Karnovsky's fixative (Karnovsky, 1965). All fixations were conducted at 4°C. After washing in cacodylate buffer, samples were postfixed in cacodylate-buffered osmium tetraoxide (2%), dehydrated in serial changes of ethyl alcohol, and infiltrated and embedded in an Epon-Araldite mixture (Trench, 1974) or in Spurr's low viscosity embedding medium. This sections prepared on an LKB Ultratome V were viewed and photographed with a Siemens Elmiskop I.

Estimation of algal photosynthetic rates

Algae isolated from the siphonal tissues by the method of Trench (1971a) and those voided in feces were incubated in sea water containing added $NaH^{14}CO_3$ (1.0 $\mu Ci \cdot ml^{-1}$), under approximately ambient conditions of temperature, and irradiance provided by cool white fluorescent lights. As controls, duplicate samples were incubated in the presence of DCMU (3[3,4 dichlorophenyl]-1,1 dimethyl urea) at a final concentration of 5×10^{-6} *M*. The DCMU, kindly supplied by E. I. DuPont de Nemours & Co., Wilmington, Delaware, was 2× recrystallized. After acidification and degassing to remove unincorporated inorganic ^{14}C, radioactive samples were assayed by liquid scintillation spectrometry. Appropriate corrections were made for quenching and background.

Chlorophyll was estimated after the algae were extracted in cold 90% acetone, and absorbancies measured at 664 and 630 nm. Calculations were based on the equations of Jeffrey and Humphrey (1975).

In situ *respirometry*

Oxygen fluxes were measured on *T. maxima* only. Two specimens, freshly collected from 5 m depth on the leeward side of Chinimi Island (Site Clyde), Enewetak Atoll, were used. Measurements were conducted *in situ* over 26 h periods (see McCloskey *et al.*, 1978) using polarographic electrodes and an automated recording system (Wethey and Porter, 1976a; Porter, 1980). Photosynthetic pigments were extracted in 90% acetone (20 h, dark, 5°C), extinctions were measured in 10 cm cells (Beckman Model DU Spectrophotometer), and chlorophyll *a* was estimated by the trichromatic equations of Richards (Strickland and Parsons, 1972).

Analysis of kidney concretions

Sections of kidney tissue mounted on glass slides were deparaffinized and coated with carbon. Analyses were conducted testing for Ca, Mg. Sr, P, As, and Sb, using an Electron Micro Probe Model EMX-5M120,000 (ARL, Sunland, Ca) at 15 KV. Standard apatite was used for comparison in the final analysis.

Results

Morphological aspects of the symbiosis

Light microscopy showed that in the siphonal tissues of the six species of tridacnid clams, the symbiotic dinoflagellates are located within the haemal sinuses

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FIGURE 2. Light micrograph of a Paraplast-embedded section through the hypertrophied siphon of *T. gigas*, showing the "eye" and the associated algae. Arrows indicate clusters of iridocytes. Scale bar = 250 μm.

FIGURE 3. Light micrograph of a Paraplast-embedded section through the hypertrophied siphon of *T. crocea*, showing the algal symbionts in the haemal sinus. Scale bar = 30 μm.

(Fig. 1). Although in certain instances one may get the initial impression that the algae concentrate around the "lenses of the eyes" (see Stasek, 1966, and Fig. 2), observations on 3–10 specimens of each species showed that this is not the case. The relative density of zooxanthellae associated with the eyes, compared to other regions of the siphonal tissue, varies from sample to sample (*cf.* Yonge, 1936; Fankboner, 1981).

Light microscopy of Paraplast-embedded tissues and of 1-μm-thick Epon or Spurr-embedded tissues provided no evidence that the algae in the haemal sinuses were inside cells of any kind (Fig. 3). These observations were corroborated by ultrastructural observations (Fig. 4), which show the algae lying free in the haemal sinuses, but sometimes adhering to the sinus walls (*cf.* Goreau *et al.*, 1973). Algae isolated from the siphonal tissues by blending or mincing the tissue with a razor blade were never found in blood amoebocytes or any other cell when examined by phase contrast or Nomarski interference optics.

The symbiotic algae in tridacnids conform in their ultrastructural characteristics *in situ* (Fig. 4) to descriptions of *Symbiodinium* (= *Gymnodinium*) *microadriaticum* (Taylor, 1968, 1969, 1974; Kevin *et al.* 1969; Schoenberg and Trench, 1980a, b, c). Although there is confusion in the literature concerning the appropriate binomial for this organism (see Loeblich and Sherley, 1979; and Schoenberg and Trench, 1980b), we have selected the binomial suggested by Freudenthal (1962), based on arguments set forth in Trench (1981).

Within the haemal sinuses, we saw many algae undergoing binary fission. Surprisingly, we occasionally found obviously pycnotic algae (Fig. 5) in the haemal sinuses, but these algae were not contained within blood amoebocytes. The interpretation that pycnotic zooxanthellae indicate host digestion is unwarranted here (*cf.* Trench, 1974). Often, one or two pycnotic zooxanthellae were observed juxtaposed to morphologically intact zooxanthellae, all lying free in the haemal sinuses.

Movement of algal symbionts from siphonal tissues to rectum

Tridacnids collected and maintained in aquaria released brown fecal pellets within 24 h of collection. Light microscopy revealed many apparently intact zooxanthellae in these pellets, accompanied by unrecognizable debris and some material recognized as crustacean exoskeletal fragments. Clams maintained in aquaria continued to release fecal pellets with zooxanthelae for more than a month after collection.

To test the possibility that defecated algae were derived from pseudofeces, 15 clams were freshly collected, and 5 were fixed immediately in Bouin's fixative. For up to 2 weeks, five clams were maintained in aquaria with running unfiltered sea water and five in 0.22 μm Millipore-filtered sea water which was changed daily. Specimens were either fixed intact in Bouin's fixative; or dissected, and the stomach, digestive gland, intestine and rectum removed and prepared for light and electron microscopic analysis. Released fecal pellets were collected and treated in a similar manner.

Light microscopy of freshly collected intact clams revealed zooxanthellae in the lumina of the tubules of the digestive gland (Fig. 6), the stomach, the style sac, the intestine (Fig. 7), and the rectum. The algae appeared intact and many were dividing. Degraded pycnotic algae were also recognized, but zooxanthellae were never seen in the mantle chamber.

Examination of the feces released by freshly collected clams revealed that zooxanthellae and unrecognizable debris, probably mostly of plant origin (Fig. 8),

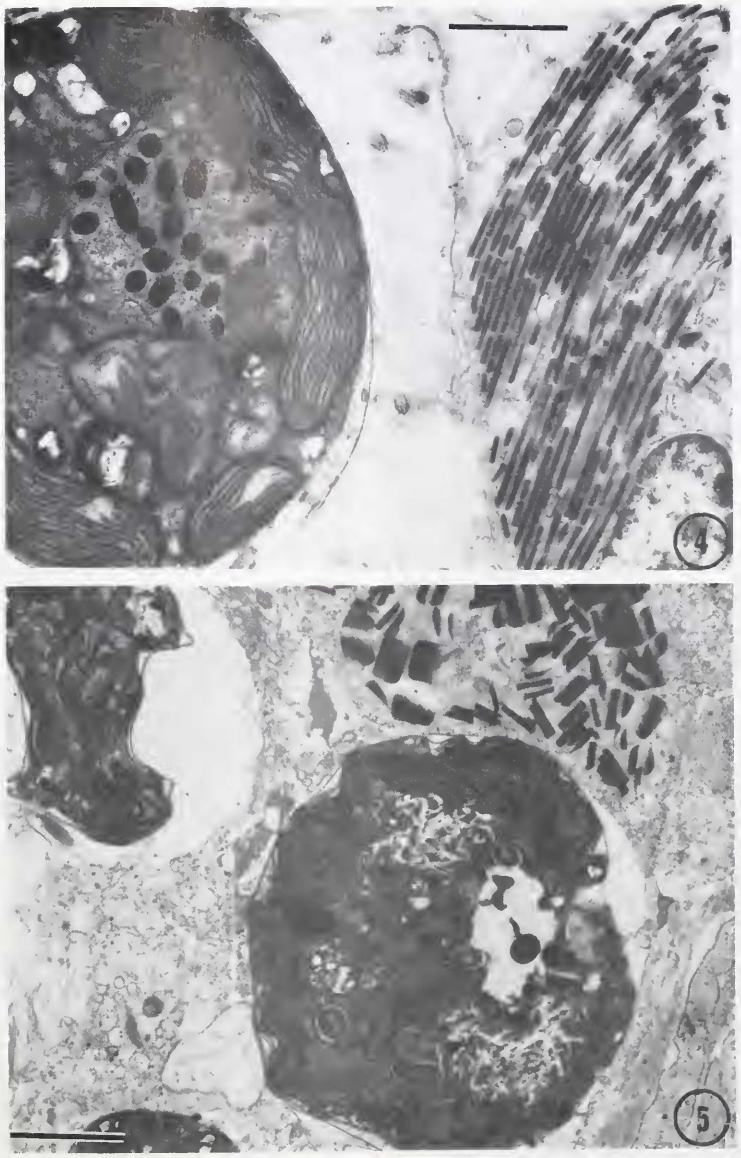

FIGURE 4. Transmission electron micrograph of *S. microadriaticum in situ* in the haemal sinus of *T. maxima*. The right hand side of the photograph shows an iridocyte which serves as a topological marker indicating siphonal tissue. Scale bar = 2 μm.

FIGURE 5. Transmission electron micrograph of two pycnotic *S. microadriaticum in situ* in the haemal sinus of *T. maxima*. An iridocyte is also shown. Scale bar = 2.5 μm.

were the major constituents. When animals were maintained in running sea water aquaria, the composition of the fecal pellets did not alter appreciably. However, when animals were maintained in filtered sea water, within 2–3 days the feces were composed almost exclusively of zooxanthellae (Fig. 9). Again, no zooxanthellae were seen in the mantle chamber. These observations suggest that the zooxanthellae

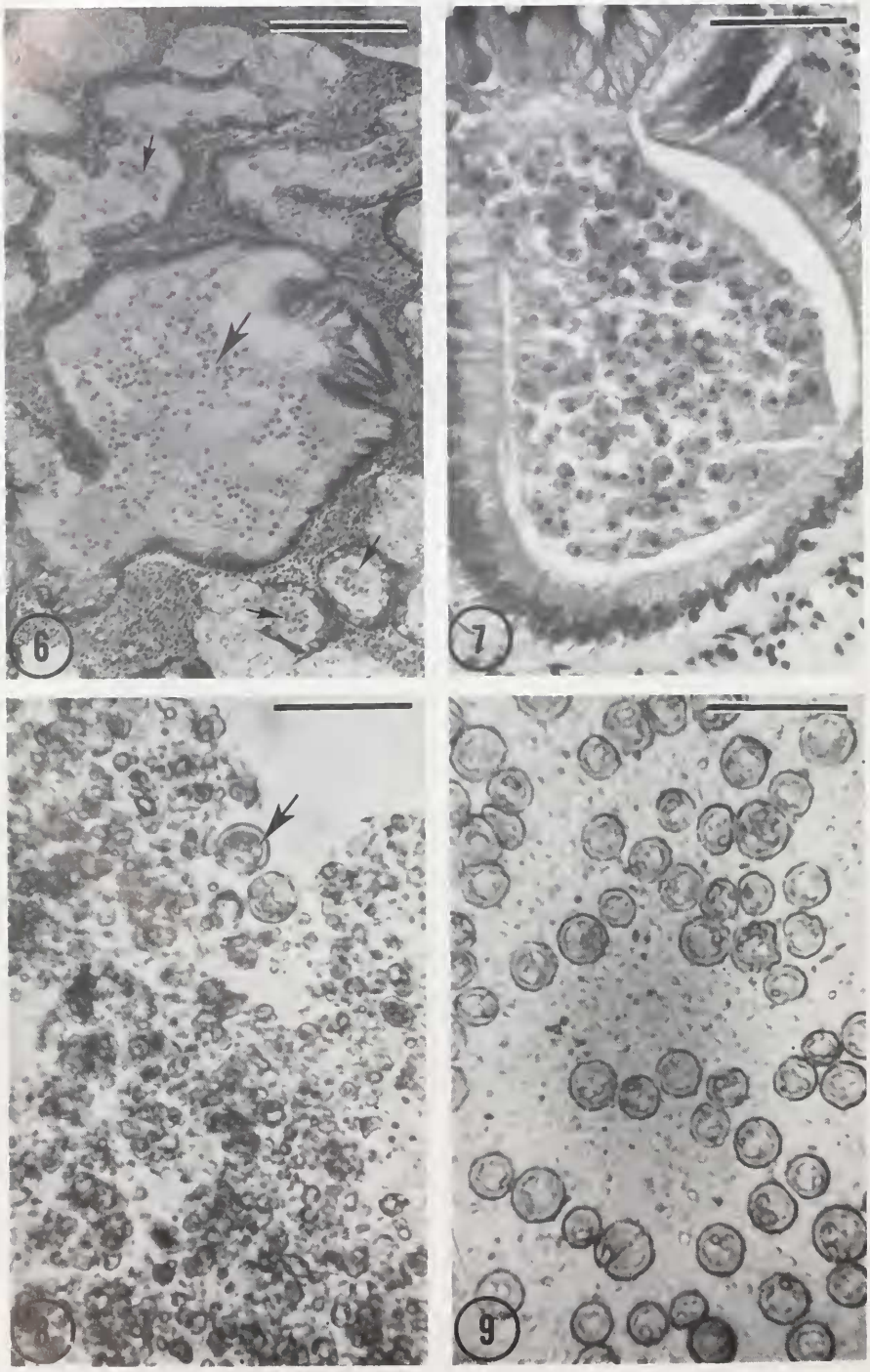

FIGURE 6. Light micrograph of a Paraplast-embedded section through the digestive diverticulum of *T. maxima*. Zooxanthellae can be seen in the lumina of the tubules of the digestive diverticulum (small arrows) and in the major collecting duct leading to the stomach (large arrow). Scale bar = 250 μm.

in the fecal pellets are not derived from pseudofeces and in addition indicate that the zooxanthellae in the feces are derived from the clam itself.

Electron microscopy (Fig. 10) confirmed the light microscopic observations, showing morphologically intact and pycnotic zooxanthellae, accompanied by unidentified debris and vesicles containing amorphous substances in the fecal pellets. Electron microscopic analysis of the contents of the rectum illustrated that many of the vesicles found in the fecal pellets were derived from macroapocrine secretions (Threadgold, 1976) originating in the epithelial cells lining the rectum (Fig. 11). Feces located in the lumen of the rectum contained intact zooxanthellae as well as zooxanthellae in various stages of disorganization (Fig. 12). None of these algae were found within cells. In addition to zooxanthellae, macroapocrine secretions, and debris, flagellated bacteria enclosed in vesicles (Fig. 13) were often observed.

Photosynthetic capacity of defecated symbionts

Comparisons of the photosynthetic rates of algae freshly isolated from the siphonal tissues, with those in the fecal pellets of freshly collected clams and clams maintained in Millipore-filtered sea water for 8 days, showed (Table I) that the rates of carbon fixation by the fecal pellets from *T. maxima* were somewhat less than, but comparable to, the rates of algae isolated from the siphonal tissues. However, DCMU inhibited carbon fixation in fecal pellets less than in freshly isolated algae, indicating that some carbon fixation in the fecal pellets was non-photosynthetic. The bacteria detected in the fecal pellets may be responsible for the non-photosynthetic CO_2 fixation (cf. Black *et al.*, 1976).

Similar patterns of separation of organic ^{14}C resulted from two dimensional radiochromatography (Trench, 1971a, b) of hot 80% ethyl alcohol extracts of labeled fecal pellets and algae isolated from the siphonal tissues (*cf.* Muscatine, 1973; Trench, 1971a, b). In addition, fecal pellets collected from *T. maxima, T. crocea, T. squamosa,* and *H. hippopus* produced viable cultures of *S. microadriaticum* when innoculated into the dinoflagellate growth medium ASP-8A (Ahles, 1967). These observations further support the conclusion that many of the symbiotic algae defecated by the clams are alive.

Identity of the kidney concretions

The kidneys of all six tridacnid species contained numerous concretions (Fig. 14) within cells of the tubules. The kidneys of juvenile *T. gigas* (≤20 mm in length) contained a lower density of concretions than kidneys of larger adult clams, suggesting that the concretions may accumulate with age (*cf.* Yonge, 1936).

The concretions, about 12–25 μm in diameter, appear to be irregular to spherical concentric lamellations (Fig. 15). Electron microscopy (Fig. 16) confirmed the light microscope observations and showed that the concretions are not located in vacuoles.

To test whether the kidney concretions were related to indigestible remnants of zooxanthellae (Yonge, 1936; Morton, 1978), we analyzed extracts of the excised kidneys for photosynthetic pigments or degraded pigments (*e.g.* pheophytin or pheo-

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FIGURE 7. Light micrograph of a Paraplast-embedded section through the intestine of *T. crocea* at the point where it leaves the visceral mass but before entering the pericardium. Scale bar = 40 μm.

FIGURE 8. Light micrograph of a freshly collected fecal pellet from *T. gigas*,showing two intact zooxanthellae (arrow). The animal had been collected 4 h before the faecal pellet was discharged. Scale bar = 20 μm.

FIGURE 9. Light micrograph of a freshly collected fecal pellet from *T. gigas* maintained in Millipore-filtered seawater for 9 days. Scale bar = 20 μm.

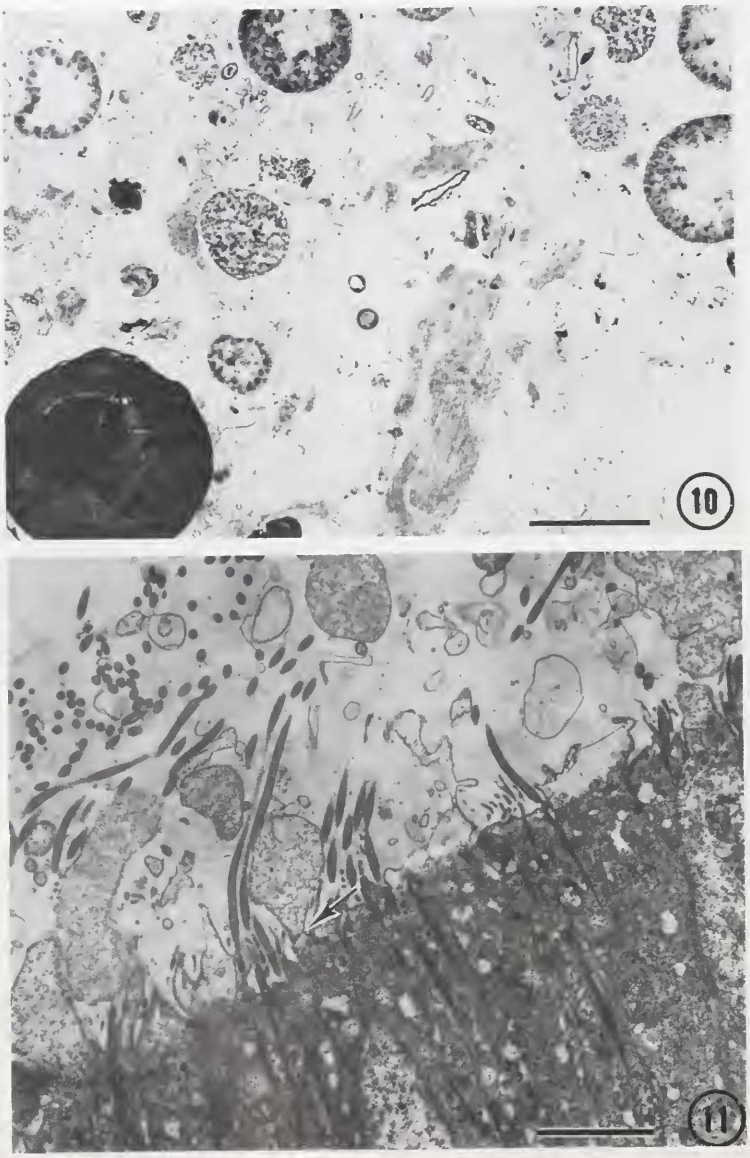
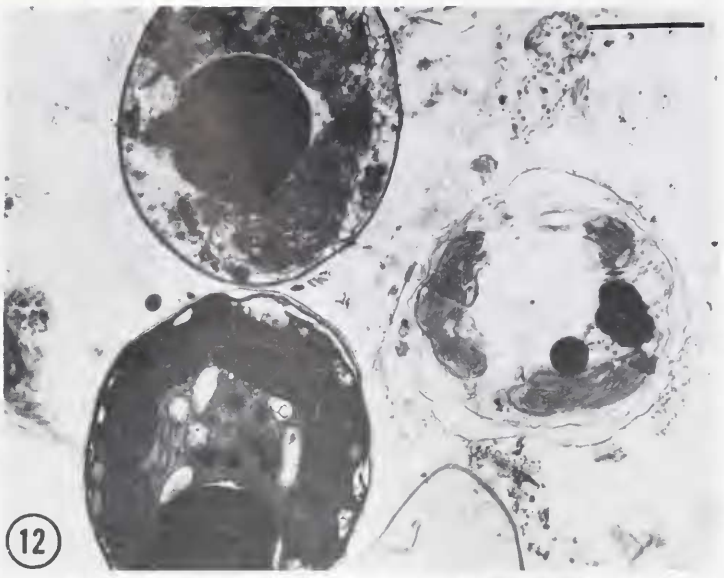


FIGURE 10. Transmission electron micrograph of a fecal pellet released by *T. derasa*. The one zooxanthella shown here appears to retain its morphological integrity. Note the many membrane-bound vesicles with amorphous contents. Scale bar = 4 μm.

FIGURE 11. Transmission electron micrograph of a section through the rectum of *T. derasa* showing the macroapocrine secretions in the lumen of the rectum and vesicles being formed but still attached to the epithelial cells (arrow). Scale bar = 5 μm.

phorbide), by spectrophotometry and by thin layer chromatography. No pheophytin or pheophorbide were detected. Minute traces of chlorophyll and peridinin were detected (*cf.* Ricard and Salvat, 1977). These probably came from the zooxanthellae often seen in vessels (probably blood vessels) that passed through the kidneys (Fig.



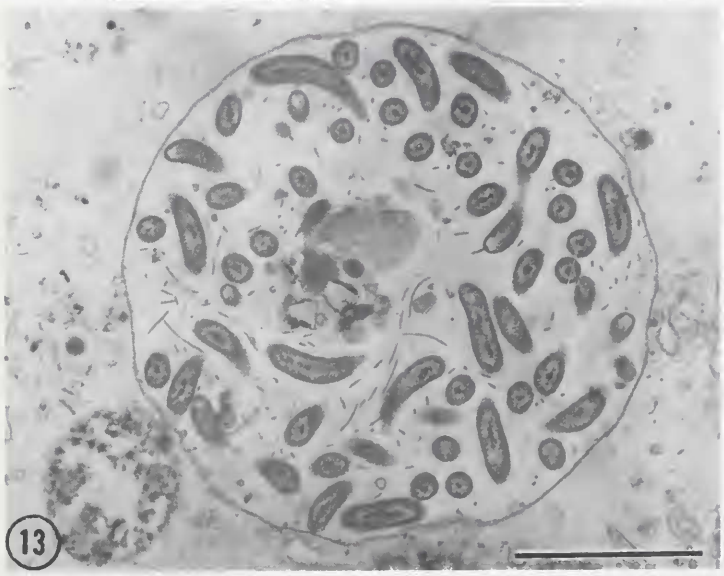


FIGURE 12. Transmission electron micrograph of a section through a fecal pellet in the rectum of *T. derasa* showing one morphologically intact zooxanthella and two pycnotic algal cells. Scale bar = 2.8 μm.

FIGURE 13. Transmission electron micrograph of a section through the rectum of *T. squamosa* showing the flagellated bacteria in a vesicle in the fecal pellet. Scale bar = 2 μm.

14), and sometimes seen also in connective tissue covering the kidneys, though never in the cells of the kidneys themselves.

Finally, electron microprobe analysis of the kidney concretions indicated the presence of calcium and phosphate in quantities similar to that found in apatite. No magnesium, strontium, arsenic, or antimony were detected. We therefore con-

TABLE I

Rates of photosynthetic CO_2 fixation by S. microadriaticum *from* T. maxima *at 25°C and 300 $\mu E \cdot m^{-2} \cdot sec^{-1}$. By comparison with Fig. 17, this radiant flux was about 50% saturation.)*

	*mg C·$(mgChl_a)^{-1} \cdot h^{-1}$	% Inhibition by DCMU ($5 \times 10^{-6} M$)
Freshly isolated algae from siphonal tissues	3.6–4.8	98–99
Fecal pellets from freshly collected animals	0.4–2.1	80–87
Fecal pellets from animals maintained in filtered seawater for 8 days	1.9–4.0	90–95

* Numbers represent range of values based on four assays involving four different specimens.

clude that the kidney concretions are biogenic phosphorite, probably homologous to phosphorite concretions found in the kidneys of other bivalve molluscs (Doyle *et al.* 1978), which do not harbor symbiotic algae.

Oxygen production and consumption by T. maxima

Two clams were used to study oxygen fluxes. The first clam had a maximum shell length of 84.8 mm, a maximum shell width of 38.5 mm, and an internal shell volume of 60 ml. The dry weight of the tissue was 4.24 g and the nitrogen content was 9.9% dry weight. The algae in the clam contained 0.38 mg chlorophyll *a*. The second clam had a maximum shell length of 87.4 mm, a maximum shell width of 45.5 mm, and an internal shell volume of 66 ml. The dry weight of the tissue was 4.85 g and the nitrogen content was 9.5% dry weight. Chlorophyll *a* content was 0.56 mg.

Figure 17 shows the net photosynthetic oxygen production of the two clams in relation to radiant flux intensity (within the photosynthetically active wavelength range 400–700 nm). The data were fitted to the Michaelis-Menten equation (Wethey and Porter 1976a; McCloskey *et al.*, 1978). Based on regression analyses of idealized curves of the photosynthesis-radiant flux relation (Bliss and James, 1966; Wethey and Porter, 1976a, b) we found an average maximum net photosynthesis (P_{max}) of 46.0 (range, 30–57) mg $0_2 \cdot$mg Chl $a^{-1} \cdot h^{-1}$. One clam produced 329.0 mg$O_2 \cdot$mg Chl a^{-1} during 12 h of daylight, and consumed 41.0 mg O_2 during 12 h of darkness, while the second clam produced 342.8 mg $O_2 \cdot$mg Chl a^{-1} and consumed 70.0 mg O_2 during the same periods. The radiant flux intensity at half-maximal photosynthetic rate (K_m) is a measure of the affinity of the photosynthetic system for light. Our estimates of K_m for these two individuals of *T. maxima* were 1.61 (95% confidence limits 1.05–2.40) and 0.94 (95% conf. lim. 0.49–1.57) Einsteins (E)$\cdot m^{-2} \cdot h^{-1}$, respectively.

To model gross photosynthesis and respiration over 24 h, we used the regression equations for the photosynthesis-radiant flux relations (McCloskey *et al.*, 1978). We recorded radiant flux with a quantum sensor on shore for 3 weeks at Enewetak and *in situ* during the experiments. We calculated the expected radiant flux at depth for theoretical sunny (maximum radiant flux 8.64 E $\cdot$ m^{-2} $\cdot$ h^{-1}) and cloudy (maximum radiant flux 4.32 E$\cdot m^{-2} \cdot h^{-1}$) days using surface recordings and measured light extinction coefficients. The theoretical daily time courses of radiant flux were divided into 1 h increments and the expected production of oxygen was cal-

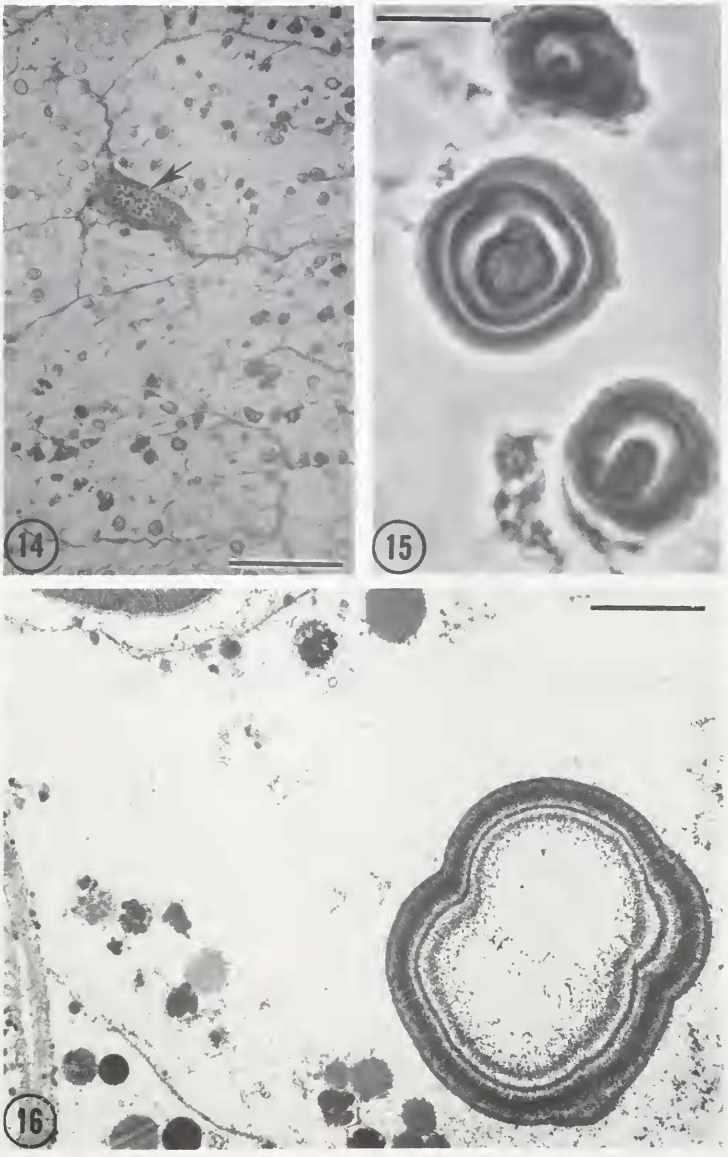


FIGURE 14. Light micrograph of a Paraplast-embedded section through the kidney of *T. crocea* showing the distribution of the concretions. The arrow indicates the "blood vessel" containing zooxanthellae. Scale bar = 170 μm.

FIGURE 15. Light micrograph of kidney concretions in *T. crocea* stained with toluidine blue. Scale bar = 15 μm.

FIGURE 16. Transmission electron micrograph of a portion of *T. gigas* kidney, showing details of the kidney cell and the phosphorite concretion. Scale bar = 9 μm.

culated for each increment using the regression equations of the photosynthesis-radiant flux relations (Wethey and Porter, 1976b). The ratio of gross oxygen production to consumption (P_{gross}/R) over 24 h was estimated as 2.02 (95% conf. lim.

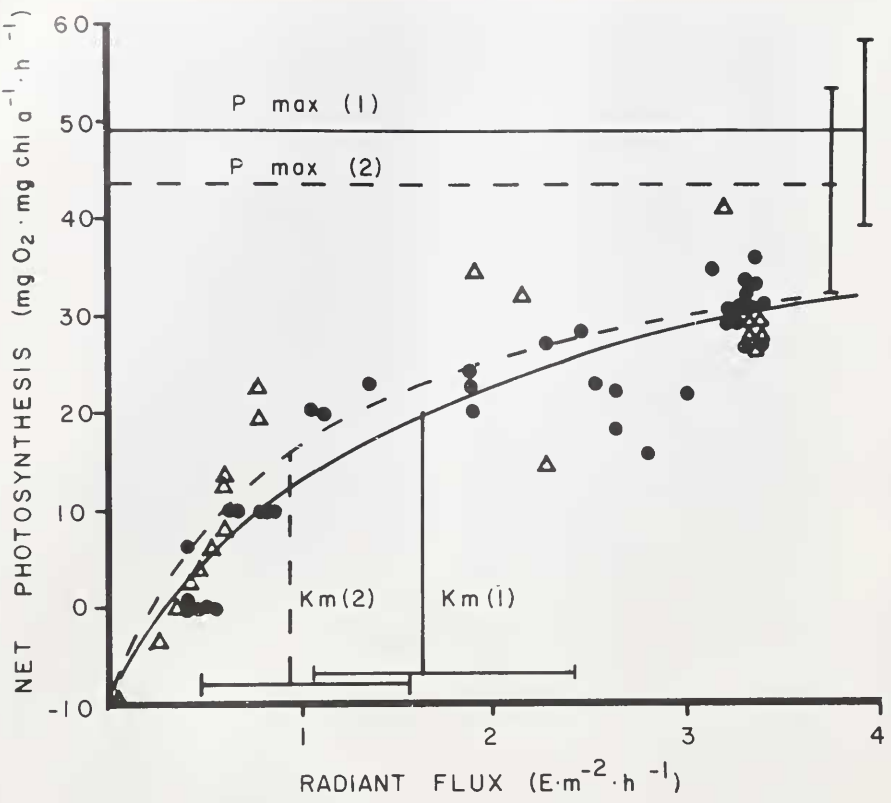


FIGURE 17. The net photosynthesis-radiant flux relation for two speciments of *Tridacna maxima* (solid circle and open triangle respectively) collected and assayed at 5 m depth on Enewetak Atoll. Fitted curves, maximum net photosynthetic rates (P_{max}), radiant flux intensities at half saturation (K_m), and their 95% confidence limits are from regression analyses of Bliss and James (1966).

1.67–2.37) and 1.86 (95% conf. lim. 1.51–2.21) for the two specimens on a theoretical sunny day, and 1.52 (95% conf. lim. 1.26–1.78) and 1.50 (95% conf. lim. 1.22–1.79) on a theoretical cloudy day.

Net oxygen production and a $P_{gross}/R > 1.0$ in plant-animal symbioses only become significant if photosynthetically fixed carbon is transported from the algae to the animals, and the animals use such translocated carbon. Muscatine (1967) demonstrated that algae from *T. crocea* released photosynthate *in vitro*. Goreau *et al.* (1973) showed the same phenomenon *in vivo* in *T. maxima*, and in addition illustrated that the translocated carbon was used in animal metabolism.

To estimate the relative contribution of algal photosynthesis to the respiratory carbon demand of the animals, we used the model of Porter (1980). In this model, the percent contribution of zooxanthellar carbon to animal daily requirements for respiration (%C) is related to the net carbon assimilated by zooxanthallae ($P_{net}z$), the percent of net carbon subsequently translocated from the zooxanthellae to the clam (% Tr), and the carbon respired by the animal (R anim), by the following equation:

$$\% C = \frac{[P_{net}z\ (24\ h)](\%Tr)}{R\ anim\ (24\ h)} \qquad (1)$$

However, oxygen flux data do not directly provide values for any of these terms. What we measure in the *in situ* respirometer is net oxygen production by the algae and the clam in daylight (P_{net} clam, daylight), and whole clam respiration at night (R clam, night). Since clam respiration is the sum of animal and algal respiration (R clam = R zoox + R anim), and since net clam production is the difference between gross algal photosynthesis and the sum of algal and animal respiration [P_{net} clam = P_{gross} zoox − (R zoox + R anim)], we need to make some assumptions to estimate the values of three variables in order to express the above theoretically accurate equation in an empirically determinable form. We assumed that a clam's daytime and nighttime respiration were equal, and that P_{net} zoox (daytime) = P_{net} zoox (24 h). The logic for these assumptions is discussed in Porter (1980) and Muscatine (1980). These assumptions allow us to calculate P_{net} zoox (24 h) and R anim (24 h) from measurements of P_{net} clam and R clam (night). We assumed an algal biomass and algal contribution to intact clam respiration of 5% (see McCloskey *et al.*, 1978; Muscatine and Porter, 1977; Muscatine, 1980), although it could conceivably vary between 1–10%. We arbitrarily gave a value of 1.0 to the photosynthetic quotient (PQ) and the respiratory quotient (RQ). Finally, using the ^{14}C technique, the measured release of photosynthetically fixed carbon by the algae from *T. maxima* was 39–45% (Trench, unpublished). We set this variable at 40%. The oxygen data were converted to carbon equivalents (McCloskey *et al.*, 1978).

Using the above assumptions and the described parameter estimates, Equation 1 can be expressed in terms of our measurable variables:

$$\%C = \frac{[(P_{net}\text{clam, 12 h day})(.375\ PQ^{-1}) + (\text{R clam, 12 h night})(.375\ RQ)(95\%)](40\%)}{(\text{R clam, 12 h night})(.375\ RQ)(2)(95\%)} \cdot 100 \qquad (2)$$

The model indicates that on a theoretical sunny day the algae may contribute 84% (95% conf. lim. 65–93%) and 77% (95% conf. lim. 63–92%), respectively, of the respiratory carbon needed by the animals. On a theoretical cloudy day they may contribute 63% (95% conf. lim. 53–75%) and 62% (95% conf. lim. 51–74%). Thus, the photosynthetic products of the algae may provide more than half the respiratory carbon requirements of the clams even on overcast days. Our measurements were made in October; in mid-summer, the contribution of the algae could be higher.

Discussion

The classic paper on the biology of symbiosis with zooxanthellae among tridacnid bivalves (Yonge, 1936) suggests, and subsequent papers (Yonge, 1953; Goreau *et al.*, 1973) reiterate, that the symbiotic algae in the tissues of the hypertrophied siphon occupy the haemal sinuses, and are "invariably contained within blood amoebocytes." These "farmed" algae were believed to be digested intracellularly by the amoebocytes in the viscera, and their indigestible remains to accumulate as concretions in the enlarged kidneys (see also Morton, 1978). Our data are inconsistent with these conclusions.

Light and electron microscopic analyses of the algae *in situ* unambiguously showed zooxanthellae in the haemal sinuses of the siphonal tissues, but no evidence of their being in amoebocytes or any other cell type was found. This is in contrast to the situation observed in several coelenterates (Trench, 1979, 1980, 1981; Trench *et al.*, in press). Our conclusion that the algae in the siphonal tissues of tridacnids are intercellular agrees with the early reports of Brock (1888), Boschma (1924), and Mansour (1946a, b, c), and with recent observations by Kawaguti (1966), Fankboner (1971), and Bishop *et al.* (1976).

Since the algal symbionts are in blood sinuses, they might be expected in other areas of the clam through which blood circulates. Indeed, Yonge (1936) observed zooxanthellae in the gills and the heart tissue, but Mansour (1946b) could not corroborate his observations. We saw zooxanthellae in the ventricle and in a vessel passing through the kidney (Fig. 14). This vessel could be synonymous with Mansour's tubular system connecting the siphon to the stomach, which Yonge (1953) discounted. We have not been able to identify Mansour's system of tubes either. The important point is that even when zooxanthellae are found in tissues other than the siphon (with the exception of the digestive gland), they are not within amoebocytes. The situation with respect to the digestive diverticulum will be dealt with elsewhere.

Obviously pycnotic zooxanthellae in the siphonal tissues (Fig. 5), often juxtaposed to morphologically intact algae, raise questions about the significance of moribund algae in tridacnids. Traditionally, pycnotic algae have been interpreted as evidence of animal digestion, usually occurring intracellularly, effected by the enzymes contained within lysosomes. However, pycnotic algae lying free in the haemal sinuses of the siphonal tissues are inconsistent with this interpretation. These algae are reminiscent of the situation in *Zoanthus sociatus*, where Trench (1974) suggested that the pycnotic algae were a result of autolytic degradation (Trench *et al.*, 1981).

In his early reports, Mansour (1946b) described intact zooxanthellae and zooxanthellae in various stages of degradation in the alimentary tract of *T. elongata* (= *maxima*). Yonge (1936) had also observed these, but concluded that they were artifactual, resulting from damage to the clams during handling.

Our study of the passage of algae from the siphonal tissues to the alimentary tract was initiated because of its similarity to the release of pellets containing pycnotic algal symbionts by zoanthids (Reimer, 1971; Trench, 1974) and live algae by sea anemones (Steele, 1975, 1977).

Since the clams continued to release viable zooxanthellae even after extended maintenance in filtered sea water, the defecated algae cannot be derived from the surrounding environment, but must be derived from the clam itself (*cf.* Fankboner and Reid, 1981). Histological sections of fixed intact clams showed no evidence of zooxanthellae in the mantle chamber, eliminating the possibility that the pellets are pseudofeces. Many zooxanthellae in the lumina of the digestive diverticulum, the stomach, the intestine, and the rectum (none of which were inside cells) were morphologically intact, but moribund cells in various stages of degradation were also observed. As the defecated algae were photosynthetically functional and could be cultured, they must have passed from the siphonal tissues through the digestive tract without being digested.

We do not know the mechanism by which the algae pass from the haemal spaces to the alimentary tract. Morton (1978) has proposed that digested zooxanthellae are discharged from the amoebocytes in the visceral mass to the lumina of the diverticulum when the epithelial lining of the tubules "break down" in a cyclical diurnal manner. We cannot deny or corroborate this, but the digestive diverticulum seems to be the most probable site where the algae enter the alimentary tract.

The evidence supporting the concept that tridacnids digest their symbionts is often inconclusive. Fankboner (1971) used the electron microscopic histochemical visualization of acid phosphatase in support of digestion of the algae in amoebocytes. However, this method does not resolve the source of the enzyme, merely the existence of enzyme activity. Trench *et al.* (1981) showed that *S. microadriaticum* possessed several isoenzymes of acid phosphatase. Hence, demonstrating acid phos-

phatase associated with algae in electron micrographs does not resolve the source of the enzyme. Our observations raise further doubt that tridacnids digest their symbiotic algae. Several independent studies indicate that bivalves are not able to digest and assimilate algal material from all sources uniformly (Coe, 1948; Dean, 1958; Haven and Morales-Alamo, 1966; and Hildreth, 1980). In addition, studies on the process of infection of juvenile *Tridacna squamosa* by *S. microadriaticum* (Fitt and Trench, in preparation) indicate that the algae enter the animals via the feeding apparatus and are somehow passed undigested from the alimentary tract to the siphon, wherein they proliferate.

The function of the macroapocrine secretions in the rectum of tridacnids is not known, but since it results in loss of cell membranes, our observations suggest that membrane turnover in the rectum of *Tridacna* may be very high. This particular mode of secretion has previously been associated with glandular tissues such as the submandibular gland of rabbits and the mammary gland (Threadgold, 1976). Although not previously reported in rectal tissue, a similar phenomenon has recently been reported in the jejunal cells of hamsters (Misch *et al.*, 1980).

The enlarged kidney of tridacnids has been regarded as the repository for the indigestible remains of zooxanthellae (Yonge, 1936; Goreau *et al.*, 1973; Morton, 1978). Our evidence does not support this conclusion. No degraded zooxanthellar pigments were identified in kidney extracts and the small quantities of chlorophyll and peridinin detected were readily traced to zooxanthellar contamination in tissues associated with the kidney. The kidney concretions are in fact phosphorite, and are common in bivalves, for example *Mercenaria* and *Argopecten* (Doyle *et al.*, 1978), which do not harbor symbiotic algae.

The existence of phosphorite concretions in the kidney of tridacnid clams raises questions on their source and mechanism of formation. It seems to us somewhat paradoxical that organisms living in phosphate-depleted environments such as coral reefs should "deposit" such large quantities of this mineral. Perhaps views on phosphate depletion in coral reef environments should be reassessed. However, *if* the phosphate is not irreversibly "locked up" in the concretions, then they could potentially serve as a reservoir of phosphorus. Obviously, such speculation needs to be experimentally tested.

The relation between net photosynthesis and radiant flux indicates that the algae in the clams living at 5 m are adapted to strong light, compared to corals such as *Pavona praetorta, Plerogyra sinuosa,* and *Porites lutea* from 10–24 m depth. The estimated K_m values for *T. maxima* were 0.94 and 1.61 $E \cdot m^{-2} \cdot h^{-1}$ as compared to values ranging from 0.11 to 0.65 $E \cdot m^{-2} \cdot h^{-1}$ for the corals (Wethey and Porter, 1976a, b). Net photosynthesis in *T. maxima* shows the same noonday peak as the corals (Porter, 1980) and shows no sign of photoinhibition even at the highest radiant flux intensities (Fig. 17).

Based on regression analyses of idealized curves of the production-radiant flux relation (Bliss and James, 1966; Wethey and Porter, 1976a, b), the P_{max} values in *T. maxima* are about five times higher than in *P. praetorta*, but the P_{gross}/R values for the two organisms are very similar, reflecting the higher animal biomass and respiration in *T. maxima.*

Since the algae produce oxygen at a high rate in the haemal sinuses of *T. maxima*, and since high levels of oxygen are potentially toxic or could cause embolisms, how is the excess oxygen removed from the system? Diffusion across the mantle surface is one possibility. Transport via the blood to the gills, where oxygen is unloaded and eliminated via the excurrent siphon, is an alternative, but this would be the reverse of the normal process of oxygen loading and unloading in

bivalves generally. Measuring oxygen tension at the mantle surface and in the afferent and efferent blood vessels associated with the gills during high photosynthesis should resolve this.

Our calculated estimates of the relative contribution of zooxanthellar photosynthesis to the respiratory carbon demands of the animals suggest that the algae represent a major source of metabolic carbon. This conclusion is provisionally supported by the studies of Muscatine (1967) and Goreau *et al.* (1973). However, the percent contribution values should not be overemphasized, because of the many different assumptions that must be made in any attempt to quantify the role of the algae in the symbiosis (Muscatine, 1980).

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